AN ESTUARINE LOW-TEMPERATURE FISH-KILL IN MISSISSIPPI, WITH REMARKS ON RESTRICTED NECROPSIES[1]

by

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ABSTRACT

In January 1973, large numbers of *Mugil cephalus* (striped mullet), weighing approximately 250 gm each, died in two freshwater localities in tidewater bayous of Jackson County, Mississippi. Fish identified as *Mugil curema, M. cephalus, Megalops atlantica, Dormitator maculatus,* and *Fundulus grandis* were found dead in other low saline estuarine areas. Fish-kills during cold periods are less commonly encountered in Mississippi than in Texas or Florida. This particular incident is attributed to conditions of stress for fishes incompletely acclimated to the encountered low temperatures. The most deleterious stress was the low saline water which probably allowed a breakdown in the fishes' ion-osmoregulatory mechanisms. Striped mullet and other euryhaline fishes in salinities greater than 6 ppt survived, as did freshwater centrarchids and ictalurids in areas with dying mullet. Other stresses thought to contribute to the weakening of striped mullet in Paige Bayou during the period of rapidly decreasing temperatures include starvation and high levels of pesticide residues. In examined fish, the alimentary tracts were devoid of food, the gall bladders were distended and leaking bile, the livers contained excess lipid material and were often stained throughout with bile pigments, and the levels of DDT metabolites and endrin residues in the liver were higher than in control fish. Stress caused by low levels of dissolved oxygen, toxic substances in the water, or disease was discounted as a cause of death.

INTRODUCTION

On 16 January 1973, following a short period of freezing temperatures, several hundred thousand dead and dying striped mullet, *Mugil cephalus* Linnaeus, were observed floating in and lining the banks of Paige and Cooper Bayous near Vancleave, Mississippi. Many times that number of fish, according to local residents, had sunk or been swept out of the area earlier that day or the previous day by the tide. Such mass mortality prompted an immediate search of other areas for dead fishes and possible causes for the deaths.

Large fish-kills occurring during cold weather, such as the one mentioned, and apparently not associated with fishing mishaps, toxic substances in the water, or low concentrations of dissolved oxygen have been documented regularly from

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Florida (Willcox 1887; Bangs 1895; Finch 1917; Storey and Gudger 1936; Storey 1937; Miller 1940; Galloway 1941; Tabb and Manning 1961; and Tabb et al. 1962) and Texas (Gunter 1941, 1945, and 1952; and Gunter and Hildebrand 1951), but not from Mississippi and Alabama. Near Corpus Christi, Texas, many people take advantage of the first cold spell by collecting stunned foodfish before the fishes move offshore. Christmas (1973:39) noted that extensive cold-kills have not been reported from the Mississippi estuaries, but that less extensive cold-kills do occur. Such deaths happen in Georgia (Dahlberg and Smith 1970) and North Carolina (Wells et al. 1961), but are rare.

I assume that the mortalities in Mississippi were caused by the combination of low temperature and other, possibly synergistic, stress-causing factors. Some of these factors will be discussed.

MATERIALS AND METHODS

Witnessing the extensive fish-kill in Paige and Cooper Bayous, tributaries of Bluff Creek in Jackson County, at Township 6 South, Range 7 West, Sections 25, 26, 27, and 35, led the writer to the inspection of other areas in the county. These included another tributary of Bluff Creek farther north in Section 16; the entrance of a canal situated parallel to Bellefontaine Beach at T8W, R8S, Section 13 and its eastern end at T8W, R7S, Section 17; Graveline Lake; a canal at T8S, R8W, Section 11, as well as Simmons Bayou in Section 2, both in Gulf Park Estates; Davis and Heron Bayous below and near the bridges of old U.S. Highway 90; Davis Bayou at the Gulf Coast Research Laboratory; the Ocean Springs Small Craft Harbor at both Shearwater and Pine Drive bridges; and Bayou Porteaux and a nearby bayou, both off Old Fort Bayou immediately north of Ocean Springs.

When dead fishes were found, as they were in some of the Bluff Creek tributaries; Simmons Bayou, a tributary of Davis Bayou; upper Davis Bayou; and a tributary of the Ocean Springs Small Craft Harbor at Pine Drive, the standard length was measured and the gills, alimentary tract, liver, and gall bladder carefully examined. Representative samples of liver and gill tissues from fish which were comatose and in a terminal state were fixed in phosphate-buffered 10% formalin for sectioning. For comparative purposes, additional material was collected alive from Davis Bayou on January 19 and 22 and August 3, 7, 24, and 29, 1973 and immediately fixed. Liver tissue was obtained both from near the base and tip of the left lobe with sections cut at 4μ or 8μ and stained using hematoxylin and eosin, Masson's trichrome method, Hall's method for bilirubin, and the periodic acid-Schiff technique with and without diastase-hydrolysis. A Sudan black B solution was used to test for lipids in frozen tissue sectioned at $10-14 \mu$ with a cryostat. The techniques followed those edited by Luna (1960).

Livers from a sample of six striped mullet from the Paige Bayou kill were immediately frozen for pesticide analysis. Samples for comparison were obtained from striped mullet caught for human consumption from Davis Bayou on 14 May 1973. Six were frozen immediately upon death and six others left at 25°C for 6 hours to reflect any differences attributable to rapid deterioration of residues of pesticide after death. Each of the three samples consisted of livers from six pooled mullet. Aliquots of 6.0 to 16.0 gm liver were added to double the amount of anhydrous sodium sulfate, 100 ml nanograde isopropanol, and 100 ml nanograde hexane, and were completely macerated in a stainless steel blender. The blended tissue was quantitatively transferred to a Buchner funnel to remove solid material. After the liquid filtrate was transferred to a separatory funnel and washed three times with distilled water to remove isopropanol, it was filtered through anhydrous sodium sulfate to remove final traces of water, and then concentrated to 5 ml in a Kuderna-Danish concentrating apparatus. Pesticide residues were then determined by gas-liquid chromatography, using a Ni^{63} electron-capture detector and calibrating with standard solutions. Analyses were performed using two different columns as a check on retention times.

Samples of water from the sites visited at the time of the fish-kill were collected and analyzed for content of salt, chlorine, and calcium. Salinity was determined with an AO Goldberg temperature-compensated refractometer; chlorosity by the low-precision, silver nitrate, titration method of Strickland and Parsons (1968); and calcium with a Perkin-Elmer atomic absorption spectrophotometer Model 305. Climatological data were obtained from monthly summaries of the U.S. Department of Commerce, Environmental Data Service, for Biloxi, Mississippi; Corpus Christi, Texas; and St. Petersburg, Florida.

OBSERVATIONS AND RESULTS

A thin sheet of ice covered the surface of Paige and Cooper Bayous during the evenings of January 13 and 14. These are sites roughly 1 to 5 m deep and completely fresh during that time of year. On the following day, vast numbers of striped mullet surfaced and died. By the next day, January 16, the tide, about 0.6 m in amplitude, removed the majority of fish, but a minimal estimation of a few hundred thousand carcasses still remained. Many had sunk and a few fish continued to surface and die. Other than mullet, the only dead fish observed were four specimens of the emerald shiner, *Notropis atherinoides* Rafinesque, 16 to 76 mm SL (averaging 71.0 mm) which had apparently been killed by the propeller of an outboard motor. "Shellcracker," "bream," "bass," and "catfish" actively fed when local residents, who frequently fed them, placed food in the water. Because of the presence of apparently healthy centrarchid and ictalurid fishes which would have also been affected by toxic materials, analysis of the water for such substances was not performed.

Additional areas nearer the coast were then inspected for dead fishes. These areas, with corresponding observed dead and living fishes, and with values for salinity, chlorosity, and calcium in the water, are listed in Table 1. Only the low-saline areas of Davis Bayou, the Small Craft Harbor, and Simmons Bayou contained dead fish. Davis Bayou was visited after most of the dead fish apparently had been swept away: the tidal flow was strong, and the remaining fish, all striped mullet, were lining the banks. Signs of raccoon and birds surrounded many partially-eaten fish. The Pine Drive area of the harbor, mostly less than 2 m in depth, had much less water movement, and the majority of dead fish had sunk. In contrast to the presence of

Table 1.

Some Characteristics of Water and Fauna on the Morning of 16 January 1973 at Several Localities in Jackson County, Mississippi

Locality	Relative amount of dead fish	Living estuarine fishes observed	Salinity ppt	Chlorosity gm chloride/liter	Calcium ppm
Paige Bayou	Very numerous	None	0.0	0.30	6.5
Upper Davis Bayou	Several	. . .	5.0	3.48	77
Simmons Bayou	Many	. . .	Low		
Small Craft Harbor Pine Drive	Several	None	6.0	4.45	94
Unnamed tributary of Old Fort Bayou	None	. . .	7.5	5.48	175
Bayou Porteaux	None	Striped mullet, spotted seatrout, and others	8.0	5.86	138
Heron Bayou	None	Cyprinodontidae	8.5	5.16	122
Graveline Lake	None	Striped mullet and others	9.0	5.45	128
Canal entrance at Bellefontaine Beach	None	Cyprinodontidae and Poeciliidae	10.0	6.68	145
Gulf Coast Research Laboratory Pier	None	Many species	10.5	6.47	141
Small Craft Harbor Shearwater Bridge	None	White mullet and others	11.0	6.99	126
Canal in Gulf Park Estates	None	Cyprinodontidae and others	14.0	8.44	165
East end of Canal at Bellefontaine Beach	None	Cyprinodontidae and others	14.0	8.99	185

exclusively dead *M. cephalus* in the mentioned areas, the harbor contained dead individuals of the white mullet, *M. curema* Valenciennes; tarpon, *Megalops atlantica* Valenciennes; and fat sleeper, *Dormitator maculatus* (Bloch); and no observed living fishes. An estimated 500 fish had died, possibly all that had been present, and other animals had been feeding on the carcasses. A few hundred meters southwest of this area, where the salinity was higher, white mullet and cyprinodontids swam near the piers. Mullet from the latter area appeared partially dazed the previous day. A few dead *M. cephalus* and one Gulf killifish, *Fundulus grandis* Baird and Girard, were collected from Simmons Bayou in low-salinity water, but, according to fishermen, many dead fish were there earlier. Numbers and measurements of the collected fish appear in Table 2. An average-sized *M. cephalus* weighed about 255 gm (0.56 lb).

Table 2.

Fishes[1] Dead in Conjunction with Low Temperature on 16 January 1973 in Jackson County, Mississippi

Area	Species	No. fish critically examined	Standard length in mm Range	 Average
Paige Bayou	*Mugil cephalus*	25	170–278	231.0
Upper Davis Bayou	*Mugil cephalus*	5	219–253	230.4
Simmons Bayou	*Mugil cephalus*	13	203–261	232.5
	Fundulus grandis	1	83	
Ocean Springs	*Mugil curema*	22	86–154	113.4
Small Craft Harbor	*Megalops atlantica*	6	226–289	251.5
Pine Drive Bridge	*Dormitator maculatus*	21	40–102	67.0

[1] The largest and smallest observed specimens of all species were included in each sample.

There were mullet and other fishes in some of the areas that were not associated with mortalities. Fishermen caught striped mullet during and after the cold weekend of January 13–14 in Graveline Bay and Bayou Porteaux. Table 1 lists other observed fishes.

During the cold weather, striped mullet in the relatively high salinity of Mississippi Sound congregated nocturnally about the mouths of bayous and spread out on the mud-flats during the day. According to a commercial fisherman, few, if any, of the mullet present in Davis Bayou on the night of January 14 fed. He said he cut more gall bladders than usual while cleaning these fish, suggesting enlarged bladders. By the following night, he reported, normal feeding behavior resumed. An examination of 13 of those fish refrigerated for 2 days confirmed an adequate intake of food: a full alimentary tract, normal-appearing gall bladder and liver, and several different parasites, all features which contrast greatly with the condition of mullet from the more freshwater habitats on the following day.

Numerous representatives of dead and dying fish from all areas were examined. The gills of dying fish looked healthy; they were filled with well-oxygenated blood and were not coated with excessive mucus. The viscera had several aspects in common: in specimens of each species, all stomachs and intestines were empty, gall bladders were greatly distended, portions of livers and often other organs were stained with leaked bile, liver parenchyma was pale, and mesenteric and other visceral depot fat was extensive. It should be emphasized that the liver's parenchyma was often stained throughout in the comatose mullet. All of the tarpon, an infrequent inhabitant of modern-day Mississippi bayous, appeared thin. Figure 1 illustrates viscera from a striped mullet that had recently died. Mullet examined 3 October 1973 also had empty alimentary tracts, enlarged gall bladders, and small pale areas in the livers (Fig. 2). Mullet, all ripe and nearly ready to spawn, a condition also apparently associated with a lack of feeding, remained void of all but a minimal amount of food throughout October and the first part of November. Figure 3 shows the viscera of a fish from December 4 that had been feeding.

Histological sections of gills and livers from striped mullet involved in the kill were compared with tissues collected 6 days and then again 7 months later. The gill tissue of the former was not diseased, had little mucus, and had far fewer ciliate protozoans and no monogeneans and copepods, all of which were common parasites in material from Davis Bayou where there were no mortalities. Sections of liver stained with hematoxylin and eosin and with Hall's method for bilirubin revealed no conspicuous bile pigments nor pathological changes in the hepatic ducts that differed greatly among dying mullet and others collected in January and August, even though bile pigments discolored the parenchyma throughout much of the livers of the dying fish. Extensive cytoplasmic vacuolation, however, did occur in the hepatocytes of the dying fish (Fig. 4). Vacuolation, to a lesser extent, appeared in all livers with the least amount found in a fish caught 24 August (Fig. 5). Using routine staining procedures, the extraction of either lipids or glycogen can cause vacuolation. Frozen sections retain lipids and some glycogen usually remains in standard sections.

Lipid material, but not glycogen, was exhibited in hepatocytes of the fish from the mass mortality. Preparations positive for Sudan black also occurred for two of three livers of fish collected August 3 and 7 that were originally suspected as being excessively fatty by gross examination. Negative stains for fat resulted for livers of the remaining fish collected January 19 and 22 and August 3, 7, 24, and 29. The Sudan black stained material throughout the entire liver, but more abundantly in the subcapsular parenchyma, in the dying fish (Fig. 6) and appeared only on distinct portions of the other two. Figure 7 shows vacuolated tissue without lipids and Fig. 8 shows less extensive lipid accumulation in a mullet during the summer.

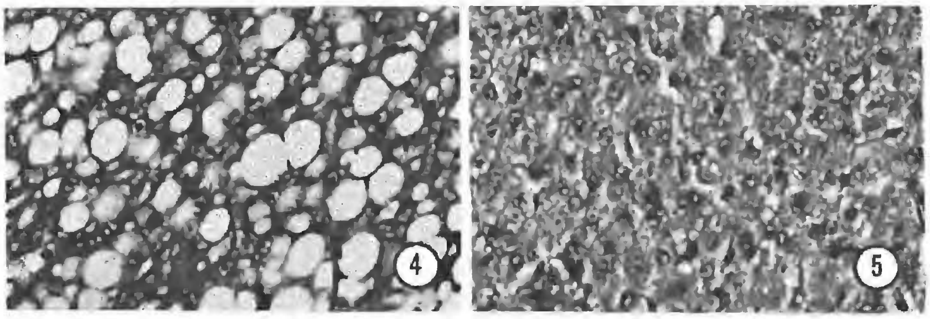
Preparations positive for PAS, hydrolyzed by diastase and thus indicative of the presence of glycogen, were demonstrated for all of the livers not collected from the fish-kill except two in August. One of those had the least amount of vacuolation of all livers. Figure 9 shows the absence of glycogen in liver from a dying fish and Figs. 10 and 11 show moderate and considerable deposits of glycogen, respectively.

In addition to vacuolation, the livers from the dying fish occasionally revealed foci of centralobular hepatic necrosis (Fig. 12). Such a finding was not unusual, since all livers possessed this possibly typical characteristic. Figure 13 reveals some inflammatory response associated with an hepatic duct even from fish exhibiting no

Figure 1. Viscera of recently collected dying *Mugil cephalus* from mass mortality on 16 January 1973. Note the empty appearing intestinal tract, pale fatty liver, enlarged gall bladder, and leaked bile.

Figure 2. Viscera of *Mugil cephalus* ready to spawn on 30 October 1973. Note that this fish also has an empty intestinal tract and an enlarged gall bladder.

Figure 3. Viscera of typical feeding *Mugil cephalus* on 4 December 1973. Note that the intestine contains material, the gall bladder is not enlarged, and the fat adjacent to the intestine is more abundant than on the above fish. The livers of some individuals at other periods may be more reddish in color.



Figures 4–5. 4. Extensive vacuolation in hepatocytes of *Mugil cephalus* dying on 16 January 1973, high power view, Masson's trichrome method. 5. Minimal vacuolation in hepatocytes of *Mugil cephalus* on 24 August 1973, high power view, Masson's trichrome method.

other pathological lesions. More pronounced was the inflammation of specific vessels in fish caught in August and containing excessive amounts of fat (Figs. 14–16). In a few sections of livers, but not those from the dying fish, a pigment, apparently a fixation artifact, surrounded some portal veins and occasionally other vessels (Fig. 17). Hepatic parenchyma of striped mullet, not in distinct lobules, were arranged in dual-plated muralia, especially notable in Fig. 8, separated by sinusoids. These sinusoids typically appeared more conspicuous peripherally.

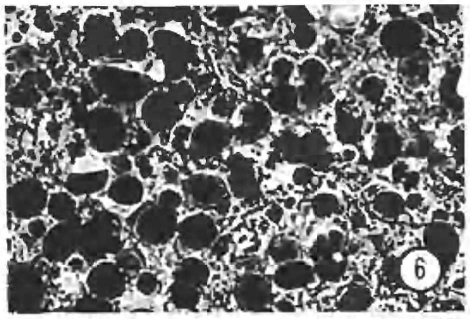
Residue levels of some chlorinated hydrocarbon insecticides appear in Table 3. Livers from *M. cephalus* dying during the kill possessed higher concentrations of endrin, p,p'–DDE, and p,p'–DDD than livers collected later from apparently healthy individuals. Allowing for individual variation, little difference existed between pooled livers of freshly caught and decomposing mullet.

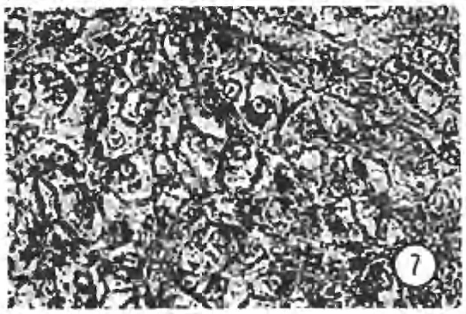
Table 3.

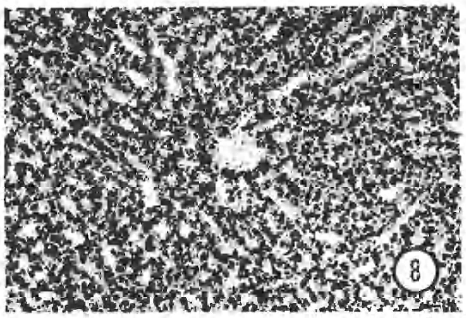
Pesticides in Parts per Million (milligrams per kilogram) Recovered from Livers of Striped Mullet Collected 16 January and 14 May 1973 in Jackson County, Mississippi

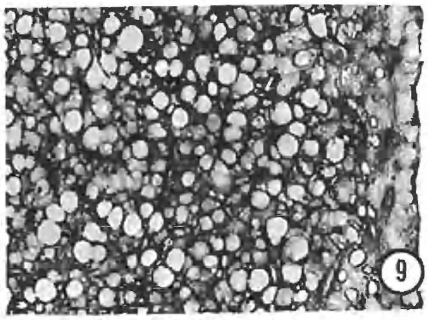
	Source of mullet		
Insecticide	Fish-kill Paige Bayou	Fresh Davis Bayou	Aged 6 hours Davis Bayou
Chlordane,α-isomer	0.44	0.73	0.38
Endrin	1.02	0.52	0.39
p,p'–DDT	0.38	0.29	0.33
o,p'–DDT	0.51	0.60	0.35
p,p'–DDE	0.64	0.33	0.32
p,p'–DDD	1.58	0.53	0.73

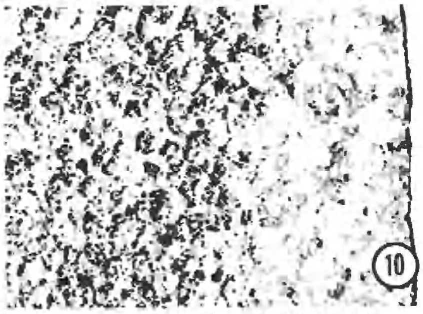
Examination for internal and external parasites revealed few organisms. The fishes from the Small Craft Harbor, somewhat decomposed, had no obvious parasites. Dying striped mullet from Paige Bayou could be examined much more

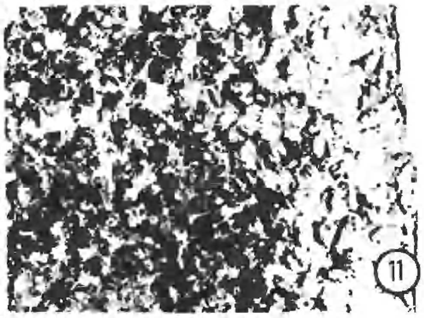






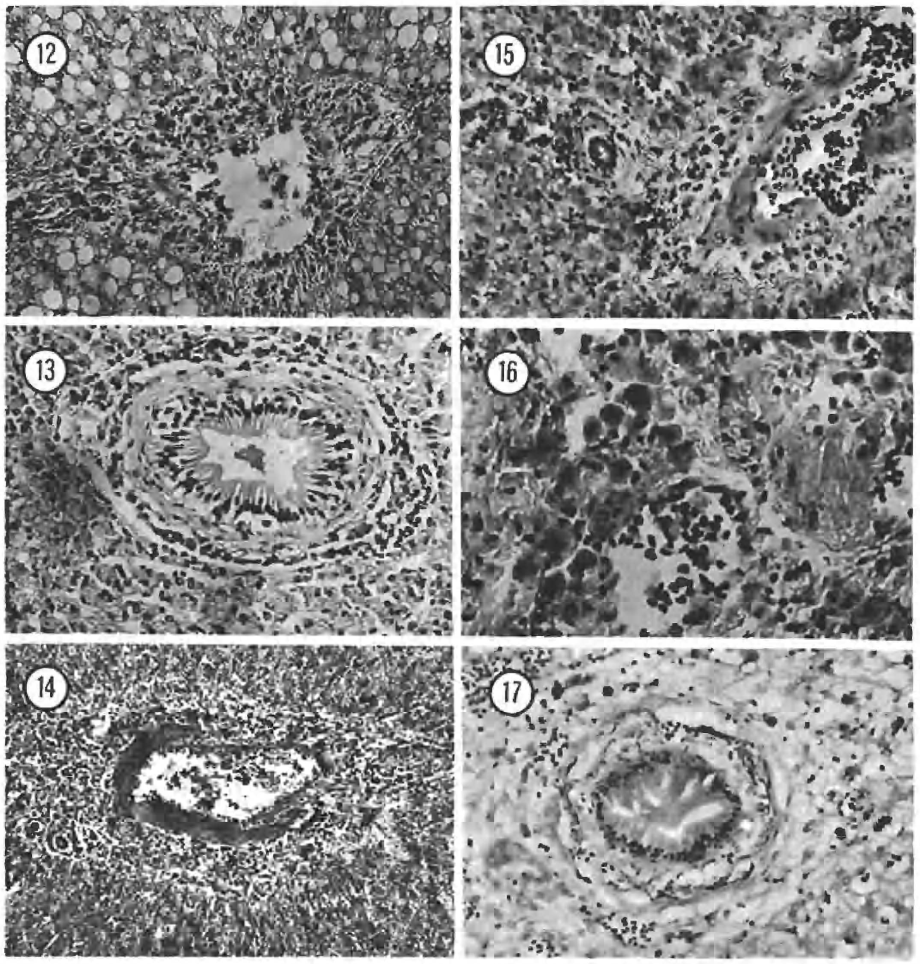






Figures 6–8. Frozen liver tissue from *Mugil cephalus* stained in Sudan black B solution to test for lipids. 6. Extensive lipid deposition in fish from 16 January 1973 kill, high power view. 7. Vacuolated tissue not containing lipids in fish from brackish water on 22 January 1973, high power view. 8. Fatty tissue from fish on 7 August 1973, medium power view. Note dual-plated muralia radiating from central vein.

Figures 9–11. Peripheral areas of liver tissue from *Mugil cephalus* stained using periodic acid-Schiff technique. 9. Liver of fish from kill on 16 January 1973 revealing no glycogen deposits, medium-high power view. 10. Liver from 7 August 1973 revealing moderate glycogen deposits, medium-high power view. Darkly stained areas representing glycogen were hydrolyzed with diastase. 11. Fish of 22 January 1973 revealing extensive glycogen deposits, medium-high power view.



Figures 12–17. Signs suggestive of pathological changes in liver tissue of *Mugil cephalus*. 12. Necrosis around central vein of fish from 16 January 1973 kill, medium-high power view, hematoxylin and eosin. 13. Minimal white blood cell involvement around hepatic duct of least pathologically altered liver examined, from fish on 24 August 1973, medium-high power view, hematoxylin and eosin. 14. More severe degeneration and inflammation within portal area of liver from 3 August 1973, low power view, Masson's trichrome method. 15. A less inflamed area of same tissue as Fig. 14 with moderate lymphocytic infiltration, medium-high power view. 16. High power view of still another area in the liver illustrating extensive involvement by eosinophils, the large cells surrounding the vein which contains smaller lymphocytes and erythrocytes, hematoxylin and eosin. 17. Substance, apparently a fixation artifact, surrounding vessel of fish from 29 August 1973, medium-high power view, hematoxylin and eosin. Similar pigment surrounded occasional veins, arteries, and hepatic ducts of a few other mullet.

critically, but harbored few parasites. Externally, the mobiline peritrich *Trichodina* sp. lightly infested the gills of most, and the piscicolid leech *Myzobdella lugubris* Leidy, 1851 occurred feeding on about 5% of several hundred superficially examined fish. One such fish had a light infection of *Trypanosoma* sp., a protomonad flagellate infecting the blood, but blood samples of the other mullet were not collected. The neoechinorhynchid acanthocephalan, *Floridosentis elongatus* Ward, 1953, infected one of ten fish examined for intestinal parasites.

On January 25, residents along Cooper and Paige Bayous reported that several dead mullet were again present on the surface. By the time the area was visited, a few thousand mullet floated on the surface, and others could be seen dead on the bottom. Examination revealed them to be bloated and well decomposed. The adipose eyelids of the fish had become translucent with hemorrhaging at the base, and filamentous algae, up to 4 cm long, covered the external surface of many fish. I believe all the floating fish had been sunken fish on January 15 and had filled with gas from decomposition and then surfaced. Healthy mullet had by this time partially repopulated the bayou. That same day, assistants examined sites of other kills, as well as additional sites, without finding any trace of dead fish.

Earlier, on January 22, a resident living on a canal off Mary Walker Bayou in Gautier, Mississippi, reported that about 3000 dead mullet had floated into his canal, but not adjacent ones, during the previous three days. These mullet were also bloated and contained considerable filamentous algal growth. They probably died earlier in the low-saline western region of Mary Walker Bayou. Personnel at nearby fishing camps northwest of the U.S. Highway 90 bridge over the West Pascagoula River stated on January 22 that they had observed no unusual number of dead fish recently, except on the two days following the freeze on January 13 when they saw large numbers of dead mullet in the upper West Pascagoula River. Since Bluff Creek joins that river, those mullet probably constituted the same ones under study.

In order for the reader to understand relative temperature values, two figures are presented. Figure 18 reveals that the air temperature reached its lowest level, −4.4°C, on January 13, after declining steadily for 8 days. For a comparative examination of temperatures in areas producing extensive mortalities, monthly values for average and lowest air temperature in Corpus Christi, Texas; St. Petersburg, Florida; and Biloxi, Mississippi were obtained. During winter months, temperature values from Texas were usually lower than those from Florida, and therefore closer to those in Mississippi and better to compare for critical differences with those from Mississippi where fish are not as prone to mortality during cold spells. This comparison, indicated by Fig. 19, shows that both the average and the lowest temperatures during cold months were lower in Mississippi than in Texas. Air temperatures replace those for water temperatures because of unavailability of the latter data. Although slightly higher in value, air temperatures reflect the general condition of the water rather well. The winter temperature of shallow water and small bodies of water surrounded by land is slightly lower than that in deep and open waters.

On 17 December 1973, the temperature once again fell below freezing, and a thin sheet of ice covered some of the coastal bayous. All the areas where dead fish were found earlier in January were examined. The salinity of the water in those areas along the coast ranged from 9.0 to 19.0 ppt, and numerous different fishes

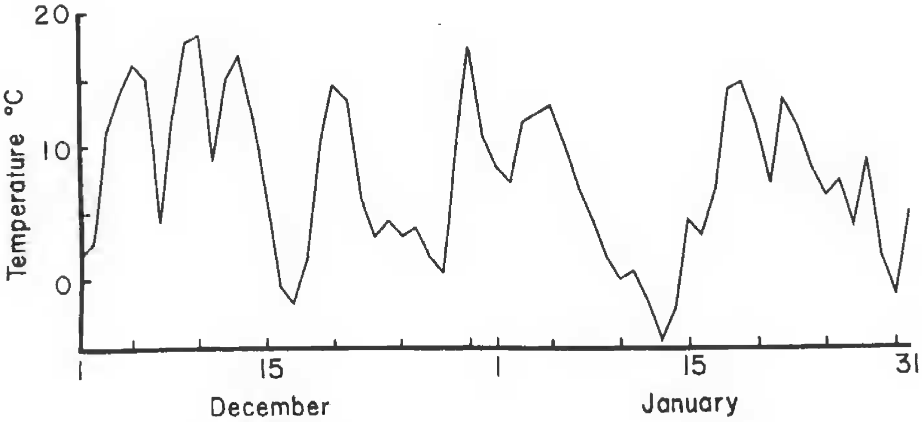


Figure 18. Values for the lowest daily air temperature during December 1972 and January 1973 in Biloxi, Mississippi.

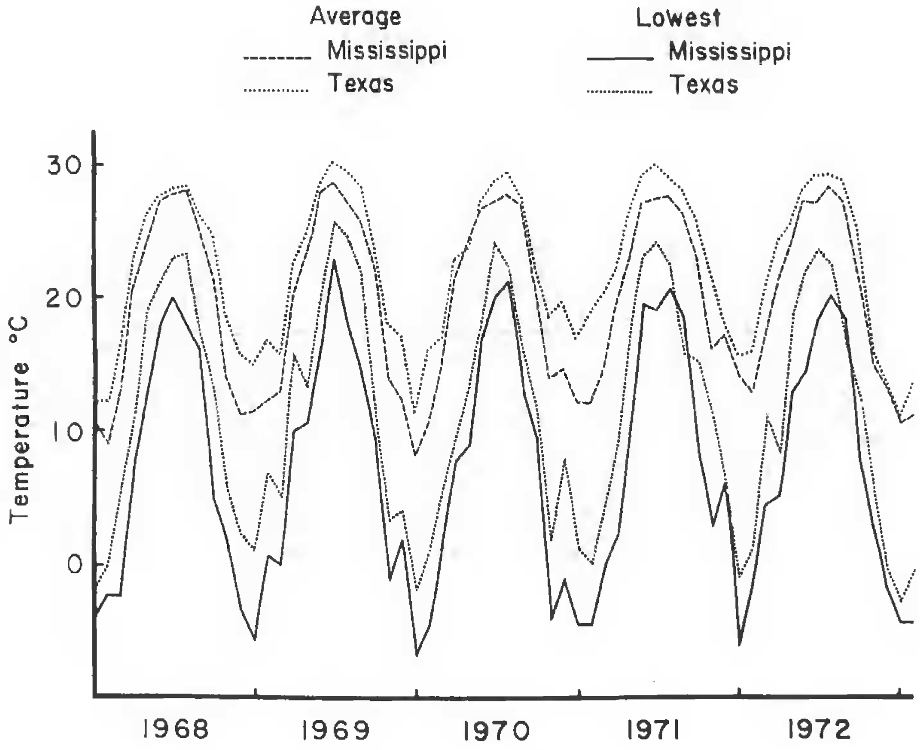


Figure 19. Monthly values for average and lowest air temperatures in Corpus Christi, Texas, and Biloxi, Mississippi, between January 1968 and February 1973.

were present. Paige and Cooper Bayous were completely fresh, but, according to operators of fishing camps, all the mullet had moved out about 2 weeks earlier during some less severe cold weather. In any event, no dead fish were observed on or following December 17.

DISCUSSION

The death of fishes reported here most certainly resulted from the low temperatures, but the cause appears to be more complicated than temperature alone. Why did the estuarine striped mullet die in Paige Bayou, while, in the same area, centrarchids and ictalurids fed eagerly, and while mullet survived in Mississippi Sound and bayous of relatively high salinity? Stress on the fish in different amounts and in addition to that caused by low temperature probably can be attributed to factors involved in osmoregulation, temperature-rate-change, acclimation, nutrition, pesticides, as well as other possibilities. Many will be discussed.

Salinity – Osmoregulation

A marine fish, such as any of the dead fishes, which is capable of entering brackish water normally counteracts osmotic and ionic changes in low saline water by regulating blood and other body fluids. Its ability to regulate osmolarity also depends on temperature. The present results suggest that at the encountered temperature or its rate of change, mullet and the other observed euryhaline fishes could not tolerate concentrations of or less than about 6.0 ppt salinity with 4.5 gm chloride per liter and 94 ppm calcium. Exact values of concentrations are probably meaningless, and presumably, if the level of calcium ions or, perhaps, other ions were higher, then salinity *per se* would have even less meaning. Calcium ions affect permeability of membranes, but their exact function remains poorly understood.

Cameron (1970) studied blood of striped mullet from the natural environment in Texas and found lowered hematocrit values, hemoglobin concentrations, and red blood cell concentrations during winter. In opposition to his finding for the pinfish, *Lagodon rhomboides* (Linnaeus), Cameron did not find a decrease in hemoglobin and hematocrit value in mullet when salinity was decreased.

Striped mullet can be transferred from seawater to freshwater, if dilution is gradual (e.g. Cummings 1955). McFarland (1965) showed that the fish regulated serum ion concentration, muscle ion concentration, and osmolarity about as well when living in freshwater as when in a hypersaline solution. Although aware of a possible influence by temperature, McFarland did not study that relationship. Also not studied in relation to temperature was the prolactin concentration of the mullet's pituitary gland (Sage 1973). Sage found that this hormone, one that apparently acts by reducing surface permeability [see review by Johnson (1973)], was involved both with initial and long-term adaptation of *M. cephalus* from seawater to freshwater. Suppression and alteration of ion-osmoregulatory mechanisms in fishes at low temperatures has been discussed (Morris 1960; Houston 1973). An alteration in acid-base balance can also accompany cold-shock (Boucher-Firley 1935). Possibly, in the kill reported here, at least a breakdown of the ion-osmoregulatory mechanism took place. I do not know how long individual mullet inhabited the freshwater bayous off Bluff Creek before the fish-kill, but the fish were devoid of many ectoparasites common in brackish areas, and a large group resided there continuously for several weeks. Immigration takes place, since a few mullet reinhabited the area by January 24.

There was no reason to suspect low concentrations of dissolved oxygen during the cold period, especially with the rich supply of oxygen in the gills of dying fish and the presence of live freshwater fishes. Results from striped mullet in Texas suggest that even if the oxygen concentration decreased, the fish could readily make appropriate physiological and behavioral adjustments to the hypoxic environments (Cech and Wohlschlag 1973). On the other hand, dissociation of any linked systems, such as ventilation and circulation, and the shut-down of any one of them, will lead to death (Roberts 1973).

Acclimation

Lack of thermal acclimation would seem to be more responsible for the reported deaths than low salinity. Both, however, are interrelated as has been suggested earlier and will be explained later. Acclimation, or the conditioning of an aquatic poikilotherm to a particular state by its previous thermal history, allows the animal to survive almost any low temperature. Brett (1960) stated "All species to date, endemic to the United States and Canada, given adequate acclimation, have shown the ability to tolerate temperatures in the region of the freezing point of fresh water. Rapidly onsetting low temperature, however, can constitute one of the greatest threats to survival when fish are accustomed to relatively warm water because of the inherent slow rate of acclimation to low temperature." The lowest temperature encountered in this study apparently preceded proper acclimation.

In addition to gradual reduction in temperature allowing for proper acclimation, it probably also allows estuarine fishes to leave unfavorable areas such as low-saline habitats. The rate of temperature decrease illustrated in Fig. 18 for the period preceding the kill had been surpassed during the previous month, but the lowest temperature had not. Fish, however, remained in near-freezing water longer in December.

Lack of proper acclimation probably determines why mass mortalities occur more frequently in Texas and Florida than in Mississippi. Figure 19 illustrates the difference in temperatures between Biloxi, Mississippi, and Corpus Christi, Texas. Fishes in Mississippi living in water normally cooler than in Texas are necessarily acclimated to lower temperatures. Consequently, a sudden drop to near-freezing levels would affect those fishes less.

The inability of tropical marine animals not accustomed to low temperatures to survive or function during such periods was discussed as long ago as 1914 (Mayer 1914). Doudoroff (1942) studied the effects of acclimation and low temperatures on marine fishes and defined two stages referred to as primary and secondary chill-comas. At low temperatures, fishes almost immediately ceased all movements and died from primary chill-coma, but could recover from the initial shock at less extreme temperatures. After some hours or days, these shocked fish again showed distress and died from secondary chill-coma. Doudoroff (1945) could delay the lethal effect at the secondary coma point by placing the fish in an isosmotic solution, apparently relieving a malfunctioning ion-osmoregulatory mechanism. Brett (1956) suggested a combination of three causes of death for young salmon in cold saline solutions slightly hypertonic to their blood: disturbance of the central nervous system, an upset osmotic balance, and a delayed unknown cause. Upset osmotic balance, as mentioned earlier, would seem to be involved in the die-off, or "secondary chill-coma," of the fishes in this study which died 1 to 3 days following the onset of ice formation on the water's surface.

Much of the mystery of acclimation and the effect of temperature on it is answered by an understanding of biochemical adaptations. Hochachka and Somero (1971) review this topic. In brief, for any given enzyme, the stabilization of its activity resulting from increases in the enzyme-substrate affinity with dropping temperature occurs only over a certain range of temperatures. Two or more variants of a given enzyme, if acting together, can then promote thermally independent enzymic functions over a wider range of temperatures. Apparently, the crucial process in cold-acclimation is the biosynthesis of enzyme variants, either polymorphisms or isozymes, better adapted for catalysis at low temperatures than the primary enzyme in the same individual. The ability for the organism's complex adaptive responses to function may also depend on the rate and extent of fluctuations of environmental parameters in addition to temperature, such as dissolved gases and salinity.

Sidell et al. (1973) measured the time course of acclimation from 15°C to 5°C and to 25°C for two compensating enzymes, and found those enzymes in short goldfish reached a steady state quicker than in large ones. In a study on metabolic rates, Morris (1965) found body-size had a significant effect on respiration rate of acclimated yellow bullhead, but that there were no differences by size in the ability to acclimate. To my knowledge, all members of every euryhaline species in the present study died. With marginal temperature-stress, however, mortalities differentiate by length. Gunter (1947) observed large individual *Anchoa mitchilli* (Valenciennes) and *Menidia beryllina* (Cope) having more difficulty surviving a natural cold-shock in Texas than small fish. Morris (1962) clarified this problem by measuring oxygen consumption of *Aequidens portalegrensis,* a native Brazilian cichlid. Stimulation with a cold-shock following cold-acclimation caused depressed respiration in fish larger than 6.0 gm only.

Nutrition

All fish examined during the period of the kill, both living and dead, possessed empty stomachs and intestines. Because of this and since their gall bladders were greatly distended with bile which leaked throughout much of the liver tissue and often on adjacent visceral tissues, I assume the fish were under some stress. Whether such stress affects the lethal thermal threshold is unknown. Under average temperature conditions, however, fish beyond postlarval stages can withstand long periods of starvation. As illustrated by Fig. 2, sexually ripe mullet occasionally stop feeding during non-winter months long enough to cause distended gall bladders and partially discolored livers. Even though I have often observed carnivorous fishes with empty digestive tracts, adult mullet, which feed mostly on plant material and sediments, typically have material in the intestine. This observation is based on examination of several hundred specimens for the presence of parasites and on comments by commercial fishermen.

Frozen sections of livers from the dying mullet revealed considerable amounts of lipid, a finding not limited to fish encountering low temperatures. The amount of permanent damage, if any, to the hepatocytes is unknown. Other than possessing relatively large vacuoles, they seemed normal.

Lipid accumulation in hepatocytes often follows periods of starvation, poisoning, or extreme stress (Hinton et al. 1973). Experimentally, it can be caused by feeding animals diets high in fat, low in protein, and deficient in lipotropic substances such as choline and lecithin. Deficiencies in vitamins A, B_1, B_6, C, E, and nicotinic acid have all been implicated in the condition. Dixon (1973) reviewed the literature concerning solubilization and cellular fatty change, including a brief discussion on the relationship between the change and temperature. Since mullet killed in the winter were starved, were osmotically stressed, and contained high quantities of insecticides, excessive fatty globulation in hepatocytes could be expected.

Using lipid stains, one can be reasonably sure vacuolation in histological sections signifies lipids rather than glycogen. Fish that had lipid-positive livers were either dying or suspected upon capture of containing copious fat from the abnormally greasy texture of their flesh and viscera. Vacuolation in livers of other fish was probably caused by glycogen that had been extracted during preparation of the sections as reported by Leske and Mayersbach (1969). Hinton et al. (1972), who positively identified glycogen as the primary cause of vacuoles in livers of largemouth bass, and others pointed out the need to critically differentiate causes of vacuolation.

Newell (1970:437) summarized the biochemical changes in liver of cold-adapted fish as extremely complex with increases in glycolysis, glyconeogenesis, glycogen synthesis, lipogenesis, and hexose monophosphate shunt participation. Both lipid and glycogen storage products may vary according to temperature, season, composition and amount of food consumed, reproductive cycle, sex, and age.

The liver and skeletal muscle serve as the main sites of lipid storage in fish, in contrast to adipose tissues in mammals (Bilinski 1969). Bilinski generalized that the liver acts as the storage site for such reserves in sluggish, bottom-dwelling fishes, whereas skeletal muscle plays this role in more active species. The striped mullet, however, from this study and additional observations appears to utilize adipose tissue as well as liver and muscle for storage. Such storage appears seasonal and seasonal variation in the amount of oil present in many fishes from the northern Gulf of Mexico is marked (Thompson 1966).

The concentration of glycogen in liver also varies seasonally and with respect to nutritional state. Swift (1955) investigated seasonal variation in food reserves of brown trout. In livers of rats, at least, the concentration of glycogen fluctuates cyclically on a daily basis and this rhythm varies throughout the year (Leske and Mayersbach 1969). Black et al. (1966) reported a 38% reduction in liver-glycogen for rainbow trout starved 84 hours when compared to controls. In hatchery-trout deprived of food for 2 weeks, glycogen and vacuolation disappeared (Simon et al.

1967). The apparent absence of glycogen in the dying mullet could be because of a starved condition.

Growth of adult striped mullet practically ceases during midwinter where studied in northwestern Florida (Broadhead 1953:23) and along the southern North American Atlantic coast (Anderson 1958). Growth may also decrease during the mullet's spawning period. Presumably during these periods, mullet utilize their reserves, suggesting that the presence or absence of vacuolation in hepatocytes represents a normal seasonal phenomenon.

Mullet also use their reserves during other periods. Cummings (1955) maintained striped mullet in a freshwater pool for 3 to 4 months after transfer from seawater. He then divided them into groups of "healthy" and "feeble" specimens and for each individual measured its blood for concentration of chloride. Those "feeble" fish had considerably less chloride and also had smaller livers with correspondingly distended gall bladders. They appeared nutritionally deprived.

A need to study seasonally large numbers of livers from male and female striped mullet, in addition to understanding the storage of lipid and glycogen, is apparent to determine the reason for the presence of the inflammation associated with many hepatic vessels. Are the various white blood cells associated with a pathological condition, caused by a natural state preceding or immediately following death, or related to haematopoiesis? Eosinophils indicate the presence of foreign proteins and lymphocytes indicate a chronic condition. Necrosis of liver cells adjacent to vessels are known to be caused by aquatic pollutants (Hinton et al. 1973).

Pesticides

Levels of pesticide residues, especially that of endrin, appear rather high in the mullet tested; these insecticides probably decreased the mullet's resistance to the effects of low temperatures. The values for both endrin and combined metabolites of DDT in livers of fish from the kill did surpass those of apparently healthy fish in May. Those for aged and fresh tissue in May agreed fairly well.

Little meaningful data exist for levels of pesticides in estuarine fishes including mullet. Hansen and Wilson (1970) reported DDT residues for homogenized whole estuarine fishes, other than mullet, near Pensacola, Florida, as rarely exceeding 0.1 ppm, except in fishes from the lower estuary in summer and fall when the amount of DDT and its metabolites reached 1.3 ppm. At least part of that residue was acquired during a spraying program. Other pesticides, including endrin, did not exceed 0.02 ppm. Alfred J. Wilson, Jr. (personal communication) analyzed several apparently healthy striped mullet from Jolly Bay near Panama City, Florida, for DDT and its metabolites. He obtained the following values recorded in ppm from pooled liver, the organ tested in this study: DDE=0.30, DDD=0.34, and DDT=0.28. All registered lower than for mullet in Mississippi. Corresponding values for residues in muscle were less, 0.14, 0.07, and 0.03 ppm. Tissues of storage such as liver parenchyma and fat typically incorporate higher levels of pesticides than other tissues, excepting possibly nervous tissues, gut, gills, and blood (e.g. Lane and Livingston 1970).

Croker and Wilson (1965) studied the effects of 0.2 pounds of DDT applied per acre of a tidal marsh near Pensacola. Small mullet, 25 to 50 mm long died first. Some pooled homogenated mullet in this length-group not killed by the pesticide accumulated 12.33 ppm DDT, and others 100 to 175 mm long possessed up to 39.24 ppm. Other mullet died after accumulating far less pesticide. When 1 pound per acre of dieldrin was applied to a salt marsh in St. Lucie County, Florida, all individuals of several species died. The striped mullet, by far the most common fish present with the possible exception of the smaller Gulf killifish, responded first to the toxic stimulus (Harrington and Bidlingmayer 1958). Endrin, found in high concentration in my study, was found to be the most toxic of 10 chlorinated hydrocarbon insecticides studied by Henderson et al. (1959). Butler (1963) listed the concentration of numerous pesticides in seawater necessary to kill 50% of tested white mullet in 24- and 48-hour periods.

The amount of pesticide that a fish can tolerate depends on numerous variables. These include genetic tolerance and environmental history. Genetically resistant stocks of several fishes are known. Some populations of mosquitofish, *Gambusia affinis* (Baird and Girard), resist over 100 times the level of endrin killing susceptible individuals (Ferguson et al. 1966). Environmental and biological parameters affect the rate, amount, and means which pesticides are presented to, accumulated by, and released from the individual. Pesticides can be acquired from food or water either quickly or slowly. Influenced by individual and species-specific biochemistry, physiology, and behavior, as well as stage of maturity and age, accumulation in the fish varies. Different organs respond differently to pesticides, and the presence of additional pesticides or chemicals may cause synergistic effects. Temperature, salinity, and, perhaps, dissolved gas may influence uptake and deposition of pesticides. A fish with a high level of pesticide in its fat and a low level in its muscle may be a healthy fish until it is starved, at which time large quantities of pesticide are released into the blood stream, killing or severely stressing the fish. The levels reported here from mullet consequently have little significance other than that considerable endrin and DDT persisted in the liver. If the deaths had been caused entirely from pesticides, residue levels probably would have been higher, behavior of dying fish would have differed, and other fishes, either in the same or adjacent areas, would have also been affected.

Governmental agencies need to perfect values for maximal permissible concentrations of residues in fish considered fit for human consumption. Mount (1967) points out that levels, unacceptable by health standards, in edible portions of fishes may result from exposure by fishes to concentrations in water that do not directly harm them. Butler (1969) presents a good review of the significance of DDT residues in estuarine faunas.

Parasites

Parasites of many kinds infect internally or infest externally the striped mullet in Mississippi. Both mullet and other species, however, examined during the kill were depauperate of parasites. On many of the mullet from Paige Bayou, a leech occurred and, although it removes blood from the fish, too few had fed to cause much stress. In addition, noninfested fish died as readily as infested ones. A trypanosome, possibly transmitted by the leech, occurred in low numbers in the blood. Even heavy infections of trypanosomes in fishes seldom severely harm their hosts. *Trichodina* sp., the ciliate, occurred on the gills in small numbers. This and related species often infest local mullet in enormous numbers, both on the gills and skin. Related species cause mortalities in fishes, but usually confined larval or young fishes such as cultured channel and blue catfishes are most seriously diseased. Post-metamorphosed flatfish in hatcheries have died from ciliate infestations (Purdom and Howard 1971); however, when mortalities occur there is usually an association of the ciliate with a monogenetic trematode (Pearse 1972). The spiny-headed worm *Floridosentis elongatus* infected too few mullet to be involved with mortalities.

The impoverished parasitic fauna suggests that it in no way affected the hosts' health. Amlacher (1961:257), however, pointed out that fishes suffering from fatty degeneration are more sensitive than are healthy ones to infectious diseases. The lack of parasites is presumably because the fish had been in freshwater long enough to cause osmotic or reproductive stress in the ectoparasites with resulting sloughing. Also, there would be no introduction of additional ecto- and endoparasites acquired from brackish intermediate hosts. Common, but conspicuously absent parasites of local mullet, include hemiurid, monorchiid, haploporid, haplosplanchnid, heterophyid, and monogenetic trematodes, an ascaridoid nematode, ergasilid copepods, an argulid, myxosporideans, and ciliates. Other parasites infect this host, but are less commonly observed in Mississippi.

Hypotheses by Local Residents

All interviewed long-time residents of the Bluff Creek area separated mortalities happening during cold periods from those caused by depletion of dissolved oxygen during warm periods. Several, however, still considered limited oxygen as the reason for the reported deaths. Residents remembered mass-mortalities in the past, but considered the present kill as one of the worst. According to a few individuals, the last two occurred in 1966 and 1969.

Since mullet died exclusively while other fishes lived, most residents attributed deaths to specific behavioral traits of the mullet. Some ascribed the cause as ruptured or frozen gill filaments from their characteristic behavior of jumping out of the water. Others suggested that the fish fed on something disagreeable such as an algae or other matter available only during cold periods or that parasites killed them.

I discount all the hypotheses. Fish had enough oxygen and their gills appeared healthy. Because the mullet had not fed, they probably did not feed on toxic substances. Toxins in the water should also kill or affect other fishes. Parasites infected fewer hosts in smaller numbers than expected and no unusual species were present. I observed no lesions or signs indicative of bacterial or viral diseases.

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